

Remarks

Claims 1-8 are pending. Claims 9-26 have been withdrawn. Claims 3 and 4 are now canceled.

Claim 1 has been amended to now recite the following:

the addition of "in" before the phrase "the presence of."
the addition of 'in vitro" before "comprising."
the addition of "wherein the NM23 acts to inhibit differentiation and maturation of said cultured cells." (previously recited in claim 3, now canceled);
the addition of "wherein the cultured cells are selected from the group consisting of stem cells and precursor cells." (previously of claim 4, now canceled).

Claim 2 has been amended to now recite "said" cultured cells.

Claim 7 has been amended to recite "consisting" instead of comprising.

All claim amendments are supported by the original specification, including the original claims.

The objection to claim 1 is believed to be overcome by Applicant's insertion of "in" before the phrase "the presence of."

The rejection of claims 1-8 under 35 USC §101 is believed to have been overcome by applicant's amendment of claim 1 to now recite "in vitro."

The rejection of claim 3 under 35 USC § 112, first paragraph, is moot as a result of cancellation of claim 3. Furthermore, claim 1 now includes the recitation "inhibit" rather than "prevents."

The rejection of claims 2-4 and 7 under 35 USC § 112, first second paragraph, is moot as a result of applicant amending claim 2 to now recite "said" cultured cells; and amending claim 7 to now recite "consisting of" instead of "comprising."

The rejection of claims 1-8 under 35 USC § 102(b) as anticipated by, or in the alternative, under 35 USC § 103(a) as obvious over Willems et al is respectfully traversed. Essentially, Willems et al. does not disclose a method "wherein the NM23 acts to inhibit differentiation and maturation of said cultured cells" nor "wherein the cultured cells are selected from the group consisting of stem cells and precursor cells." Moreover, Willems actually teaches away from Applicant's claims for the following reasons:

Willems et al. studies two populations of cells that were sorted from adult bone marrow. The first of these were CD34++CD38- cells and the second CD34+CD38+. Note that the first population has ++ after CD34 and the latter +. This is to signify that the one

population has stronger expression of this 'antigen' as revealed by a greater intensity of fluorescence when stained to measure its presence. The authors correctly state that the most primitive cells (and for our purposes including stem cells) will be present in the CD34++CD38- fraction. The other cell fraction contains so-called committed cells or progenitor cells. For our purposes this more mature population will not contain stem cells.

Given the known differences in the properties of their cell fractions Willems et al used differing assays to assess the impact of Nm23 proteins on their behavior. The stem cell fraction, that is, the fraction containing CD34++CD38- cells used what they have called their pre-CFU (pre-colony forming unit) assay. The assay involved plating out a variable number of sorted CD34++CD38- cells (ranging from 300-15000) in 96 well plates in serum free medium. This is a much lower cell density (20-50 fold) to that which is used in the applicant's experiments. Willems et al also used a much richer expansion cocktail of cytokines than in the applicant's experiments. The Applicant had used SCF and IL3, both alone and in combination. Willems et al too used SCF (which they call by its alternative name of kit-ligand) and IL3, but in addition used FL (Flt-ligand), IL1 and IL6. This cocktail of cytokines would provide a strong stimulus for proliferation and concomitant differentiation. Although the data is presented in a way that prevents us from knowing just how much proliferation they observed, note that in the materials and methods they describe analyzing numbers of expanded cells using either an inverted microscope or a haemocytometer. This means that they must have quite variable proliferation, or as they describe it, expansion (requiring a haemocytometer to enumerate the cells where there had been high proliferation).

More importantly, none of the Nm23 proteins they used at either dose affected overall cell expansion/survival of CD34++/CD38- cells as measured by cell count at the end of 14 days culture (figure 2A). This is in marked contrast to our client's own data used to support the present patent application. We show a marked increase in cell number in response to NM23H1 when IL3 and SCF are present.

In the Willems' disclosure no re-phenotyping (i. e., staining) for CD34 or CD38 was performed after the 14 days of culture in the presence of SCF, IL3, IL 1, IL6, and FL (with and without Nm23). The cells were analyzed by the CFU assay only. Therefore, we cannot know if CD34++CD38- cells were protected or otherwise in the presence of Nm23 proteins. The colony assays they use are not stem cell assays and would not reveal the presence of such. What the colony assays measure is the relative numbers of cells that have become committed within the cell population. The problem with these assays is that the cloning efficiency is always low. In their experiments the cloning efficiency (CE) in the control group (i.e., cells that had been cultured in SCF, IL3, IL 1, IL6, and FL but without Nm23) was 11% and in the additional presence of 1 ug/ml NM23H1 8%. What this means is that for every 100 cells placed in the assay only 8-11 cells give rise to colonies. The general wisdom is that this reflects the overall makeup of the population but in reality we know very little about the quality or characteristics of the other 89-92% of cells.

The key quote from the authors appears at the bottom of page 643 "no consistent and/or dose-dependent modulation of the proliferation of primitive haemopoietic progenitors by extracellular NM23 could be demonstrated with this assay. After differential scoring, the proportion of the various differentiation lineages was determined for each condition. However, comparison of these data did not reveal any statistically significant effect caused by any of the NM23 isoforms."

The second part of the paper is largely irrelevant since it regards committed cells not stem cells, however, it is worthy to point out that the data does not indicate a block in differentiation but rather a shift from one form of differentiation to another.

Thus, it is submitted that Willems actually teaches away from the invention and therefore the method according to the present invention, being a method for the inhibition of differentiation and maturation of stem cells in culture, is not disclosed or suggested by the Willems reference.

In view of the foregoing amendments and remarks, Applicant believes that the application is in condition for allowance and respectively solicits a Notice of Allowance.

The Commissioner is hereby authorized to charge payment of any fees required associated with this communication or credit any overpayment to Deposit Account No. 50-3881.

January 11, 2010

Respectfully submitted,



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